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Abstract

Heat and hypoxia exacerbate central nervous system (CNS) fatigue. We therefore investigated whether essential-amino-acid (EAA) and caffeine ingestion attenuates CNS fatigue in a simulated team-sport specific running protocol in a hot and hypoxic environment.

Sub-elite male team-sport athletes (n=8) performed a repeat-sprint running protocol on a non-motorized treadmill in an extreme environment on four separate occasions. Participants ingested one of four supplements: a double placebo, 3 $mg.kg^{-1}$ body mass of caffeine+placebo, 2x7 g EAA (Musashi Create™)+placebo, or caffeine+EAA prior to each exercise session using a randomized, double-blind crossover design. Electromyography (EMG) activity and quadriceps evoked responses to magnetic stimulation were assessed from the dominant leg at pre-, half-time, and post-exercise. Central activation ratio (CAR) was used to quantify completeness of quadriceps activation. Oxygenation of the pre-frontal cortex was measured via near-infrared spectroscopy. Mean sprint work was higher (+174J, 23–24J; mean, 95% confidence interval, d=0.30; effect size, likely beneficial) in the caffeine+EAA condition versus EAAs alone, but not caffeine or a placebo. The decline in EMG activity was less (13%, 0–26%; d=0.58, likely) in caffeine+EAA versus EAA alone. Similarly, the pre-to-post exercise decrement in CAR was significantly less (-2.7%, 0.4–5.4%; d=0.50, likely) when caffeine+EAA were ingested compared to placebo. Cerebral oxygenation was lower (-5.6%, 1.0–10.1%; d=0.60, very likely) in the caffeine+EAA condition compared to LNAA alone. Co-ingestion of caffeine and EAA appears to maintain muscle activation and central drive, with a small improvement in running performance relative to EAA alone in hot, hypoxic environments.

Key words – Repeated sprint exercise, Central fatigue, Hypoxia, Heat, Amino acids, Caffeine

Introduction

Fatigue is characterized by the inability of skeletal muscle to maintain the required power output (Allen et al. 2008), and may occur at a muscular level (peripheral fatigue) or in the central nervous system (CNS, central fatigue). Central fatigue manifests as the inadequacy to voluntarily activate muscles (Gandevia 2001), alterations in cognitive function, perception of effort, and pain (Meeusen et al. 2006). The Central Governor theory highlights the importance of the CNS and the potential role of accumulation and/or depletion of neurotransmitters in fatigue (Noakes 2011). Because neurotransmitter synthesis and function can be manipulated using nutritional strategies, it may be possible to use these strategies to reduce central fatigue (Pardridge 1998; Meeusen et al. 2006).

Caffeine is considered a legitimate nutritional supplement in competitive sport to delay the onset of fatigue. Research on the impact of EAA on fatigue, however, has been equivocal (Davis et al. 1999; Watson et al. 2004; Stepto et al. 2011). EAA supplementation did not improve performance in endurance exercise (Madsen et al. 1996; Davis et al. 1999) in normal conditions (thermoneutral, normoxic environment), but may do so in the heat when production of dopamine is augmented. (Mittleman et al. 1998; Tumilty et al. 2011) Reducing dopamine synthesis is associated with a quicker onset of fatigue (Bailey et al. 1993). To our knowledge no studies have examined the effect of EAA supplementation on exercise performance in hypoxia.

Caffeine may enhance performance in endurance exercise, (Doherty & Smith 2004; Ganio et al. 2009), high-intensity running, and sprint activity (Glaister et al. 2008; Astorino & Roberson 2010) in normal conditions. Caffeine ingestion prior to exercise can reduce ratings of perceived

exertion (RPE) (Doherty & Smith 2005), improve running power (Schneiker et al. 2006), and decrease sprint times (Glaister et al. 2008; Gant et al. 2010). Evidence for an ergogenic effect of caffeine in hypoxia is limited or equivocal when exercise is performed in the heat (Del Coso et al. 2008; Roelands et al. 2011).

Exercise in the heat and/or at altitude represents a situation of maximal environmental stress that could accelerate the development of central fatigue. Voluntary muscle activation is reduced after exercise in the heat (Skein et al. 2012), possibly related to altered dopamine activity and inhibitory signals from thermoreceptors in the hypothalamus (Nybo 2008). Similarly, voluntary activation is reduced after exercising in hypoxia (Verges et al. 2012; Billaut et al. 2013) as a result of cerebral deoxygenation (Smith & Billaut 2010; Billaut et al. 2013). Therefore, manipulating environmental conditions in combination with nutritional interventions can provide insights into novel supplementation strategies and the mechanisms of CNS fatigue.

The aim of this study was to investigate whether nutritional supplementation can attenuate central fatigue and reduce performance decrements under conditions of environmental stress (simulated heat and hypoxia). Although these conditions are uncommon, it provides an opportunity to observe the potentially beneficial effects of nutritional manipulations under standardized conditions. Nutritional supplementation, in combination with assessing markers of central fatigue (e.g., muscle recruitment, central drive), will clarify the viability of invoking changes in neurotransmitter concentration in relation to central fatigue. We hypothesized that central fatigue and decrements in intermittent-exercise performance induced by environmental

stress would be attenuated by targeted nutritional supplementation strategies utilizing a combination of both caffeine and EAAs.

Materials and Methods

Participants

Ten male, sub-elite football players regularly competing and training (~60 min, 3 times per week) volunteered for the study (Table 1). Two athletes were unable to complete the study, due to issues and/or injuries unrelated to the study. Prior to participation, each subject provided written informed consent for their voluntary participation in the study. The study was approved by the local Human Research Ethics Committee.

Participant characterisation/familiarisation

Participants reported to the laboratory 10-14 days prior to the experimental protocols on two occasions. On the first visit, age, height and body mass were recorded, body composition assessed by a whole body light scanner ($[TC]^2$, Cary, U.S.A., (Ryder & Ball 2012)), and participants were familiarized with the exercise protocol. On the second visit, participants performed a 20-m sprint followed, 30 min later, by the Yo-Yo intermittent recovery test (Mohr et al. 2003).

Diet and exercise control

Two days prior to each experimental trial participants consumed a moderate-high carbohydrate diet (4 $g.kg^{-1}$ body mass) that provided ~106 $kJ.kg^{-1}$ body mass per day (70% CHO, 15% protein and 15% fat), while controlling protein intake in line with Australian dietary recommendations.

Participants were also instructed to abstain from any caffeinated products, alcohol and formal exercise training 24 h prior to testing.

Study design

In each session, after voiding, and being weighed, athletes consumed the initial supplement (EAA or Placebo) and remained in the laboratory at normoxic and thermoneutral conditions for the first 2 hours prior to exercise (Figure 1). At the end of this period, one hour before exercise, athletes consumed the second dose of supplements (caffeine, placebo and/or EAA) and rested in the environmental exercise laboratory (15% O_2 [~2700m above sea level], 30°C and 20% relative humidity). Although this combination of conditions is uncommon, in research studies it provides a unique opportunity to invoke central fatigue under standardized conditions and observe the potentially-beneficial effects of nutritional manipulations.

Table 1: Participant characteristics

Age	22 ± 2
Height (cm)	181 ± 6
Body mass (kg)	77 ± 11
Estimated body fat (%)	14.7 ± 4.4
20m sprint time (sec)	3.1 ± 0.1
Yo-Yo IR1 distance (m)	1320 ± 528
Estimated VO2 max ($ml/kg.min^{-1}$)	47.5 ± 4.4

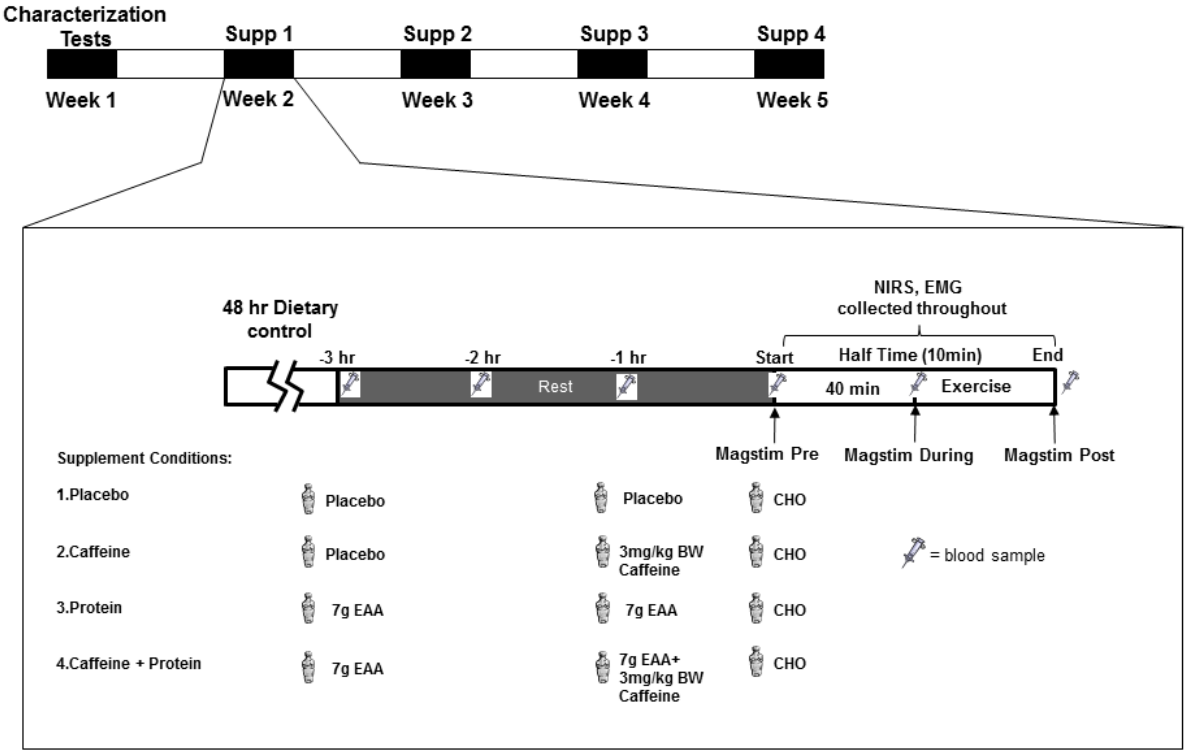


Figure 1: Schematic diagram of the study design. Supp = Supplement, Magstim = Central activation and MVC assessment, BW = Body Weight.

Exercise Protocol

The exercise protocol (Figure 2) was designed to mimic the running profile of team-sport athletes (Sirotic & Coutts 2008; Zois et al. 2013). During each session, participants completed a total of 6 sets of 4 s sprints with 8 s of rest between each sprint on a non-motorized treadmill (Woodway Force, Waukesha, U.S.A.). The time between each sprint set was interspersed with standing (22% of total time), walking (27%), jogging (22%), running (12%) and fast running (9%). Mean power, peak power and peak velocities during the exercise protocol were recorded

by the treadmill force transducer at 25 Hz. Performance data were stored and analyzed as described previously (Serpiello et al. 2011).

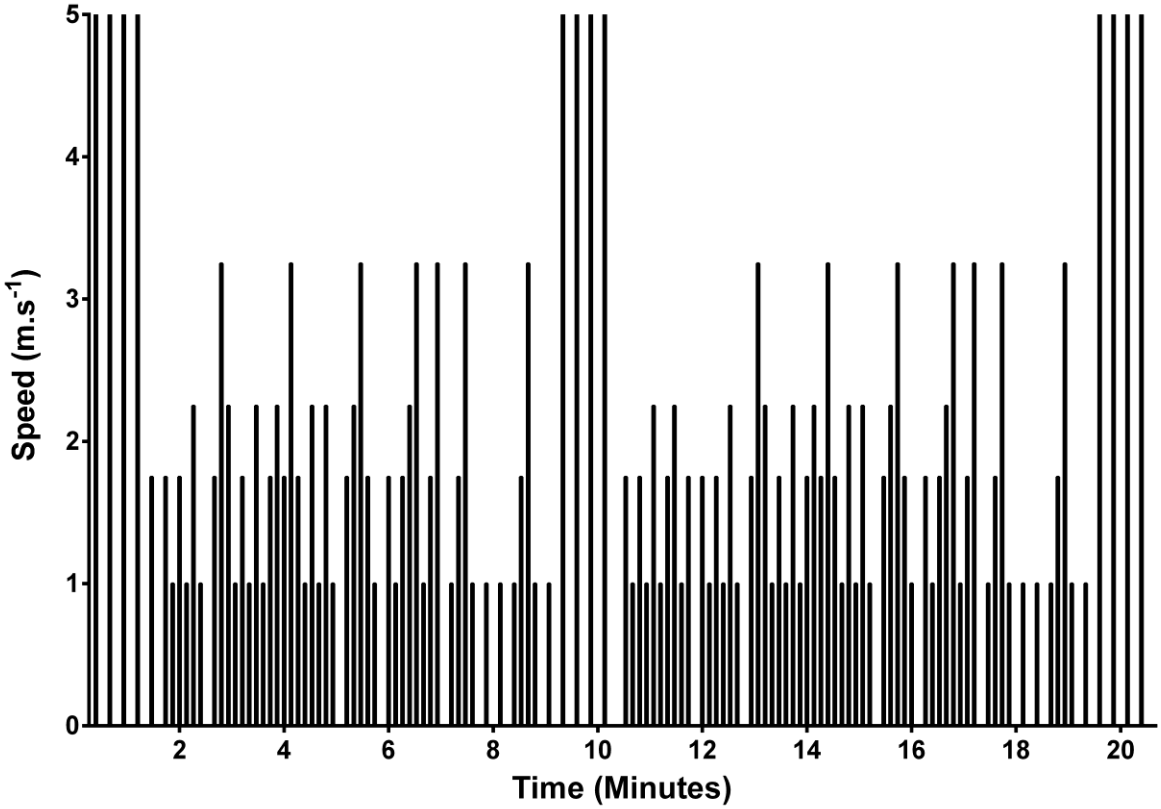


Figure 2: Graphical representation of one half of the exercise protocol, including 3 x 4 x 4 second sprints and intermittent activity for participants with a maximal sprint speed of 4.8 – 5.2 $m.s^{-1}$. This protocol was completed twice with a 10 minute break in-between to simulate half-time.

Supplementation regimens

Four supplement protocols were undertaken in a counterbalanced, randomized and double-blind fashion to provide the participants with a placebo, caffeine only, EAA only, or caffeine + EAA (Figure 1). The EAA was provided in two 7 g doses of Musashi Create™ powder, dissolved in

250 ml of water containing; arginine (854 mg), leucine (837 mg), phenylalanine (794 mg), lysine (709 mg), valine (598 mg), histidine (555 mg), isoleucine (512 mg), threonine (418 mg) and methionine (162 mg), to reduce serotonin, and increase dopamine production within the CNS. Musashi also provided an inactive placebo that was indistinguishable in color and taste. Anecdotally, participants were unable to correctly identify any of the treatments. The EAAs or placebos were given to the participants 3 h and 1 h prior to commencing exercise trials. Caffeine (to block the action of adenosine; No-Doz™; 3 $mg.kg^{-1}$ body mass) or placebo ($CaCO_3$) was given to participants as a capsule (blinded) 1 h before exercise. Immediately before the warm-up, participants also consumed a carbohydrate gel (27 g; Powerbar Gel™, Musashi Performance Nutrition, Nestle, Australia) to ensure adequate provision of carbohydrate (Figure 1).

Blood sampling

Six 5ml blood samples were taken at regular intervals in each trial (Figure 1). Baseline blood samples (3 h pre-) were taken prior to the ingestion of the initial supplement. Blood samples were analyzed immediately for partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), oxygen saturation (sO_2) (Siemens, Henkestrasse, Germany) and lactate and glucose concentrations (YSI, Yellow Springs, U.S.A).

Central fatigue assessment

The knee extension protocol used has been described in detail elsewhere (Billaut et al. 2013). Before, at half-time, and immediately after exercise, participants were instructed to perform a maximum voluntary contraction (MVC) of the quadriceps (dominant leg) against the strain gauge. In this position, a magnetic stimulator (Magstim RAPID; JLM Accutek Healthcare,

NSW) and a double 70-mm coil (producing two overlapping circular fields) were used to stimulate each participant's quadriceps during the MVC (Amann et al. 2007; Billaut et al. 2013). The central activation ratio (CAR) was calculated as described previously, where CAR=MVC/(MVC + superimposed twitch) (Gandevia 2001).

Surface electromyography (EMG) acquisition and analysis

EMG signals were recorded via skin surface electrodes over the vastus lateralis, rectus femoris, tibialis anterior, biceps femoris, gluteus maximus and lateral gastrocnemius of the dominant lower limb. In each trial, electrodes were secured to the skin with tape and an elastic tubing to prevent cable movements. In the first trial, electrode placement was marked with a permanent marker to ensure that the position of the electrodes was replicated on subsequent visits. EMG signals were collected using the Delsys system (Delsys Inc., Boston, MA) at 2 kHz after being filtered with a 50-Hz line filter. Muscle activity was quantified as the integrated EMG of the signal between the onset and offset of activation of each burst in the last 2 s of every 4 s sprint. EMG signal from all muscles were added together to provide a value representative of overall muscle activation in the lower limb. Data were normalized to the first sprint set to provide a percentage change for total electrical activity to account for a potential disproportionate effect of the first sprint.

Near-Infrared Spectroscopy (NIRS) measurement and analysis

Brain oxygenation was measured using NIRS (Artinis Medical Systems, AS Zetten, Netherlands). The protocol allowed the calculation of the tissue saturation index (TSI) for cerebral tissue (Smith & Billaut 2010; Billaut et al. 2013).

Plasma amino acid analysis

Concentrations of the free amino acids histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophanin in plasma were determined using reversed-phase high-performance liquid chromatography (RP-HPLC) as previously reported (Stepto et al. 2011). The limit of quantitation for this study was 0.5μmol.L^{-1} with intra- and inter-assay variability of 4.5% and 5.1%, respectively, for tryptophan, and <2.3% and 3.8%, respectively, for the other EAAs.

Caffeine analysis

Plasma caffeine concentrations were assessed via RP-HPLC (GBC Scientific HPLC system, GBC Scientific, Victoria, Australia) according to the method of Alvi & Hammami (2011). The internal standard was antipyrine in 30% $HClO_4$ [40 μg/ml]) A 5μm, 4.6x150mm RP C18 column and a 5μm, 4.6x20mm Guard Cartridge insert (Water Atlantis® T3) were used for the separation. The mobile phase consisted of 15 mM potassium phosphate (pH 3.5) and acetonitrile (83:17, v/v), The analysis was performed under isocratic conditions using a flow rate of 1.0 mL.min^{-1}. Chromatograms were recorded at 274 nm with a run time of 10 min. The data were collected with a personal computer using EZChrom Elite Chromatography Manager Software and plasma caffeine concentration determined using peak area ratio (caffeine to antipyrine). The assays limit of quantification in plasma was 0.05 mg.L^{-1} with an intra- and inter- assay variability of 2.5% and 5.6% respectively.

222 **Statistical analysis**

223 Data for the 8 participants are expressed as mean ± SD (see Supplementary Tables 1-4). All data
224 were analyzed using a linear mixed model of the estimated true difference between
225 supplementation protocols with Bonferroni *post hoc* adjustments used where appropriate.
226 Estimated mean differences for the fixed effects of time and supplementation strategy, and the
227 interaction of both (time × supplement) were determined for each parameter. Precision of
228 estimation was indicated with 95% confidence intervals (95%CI). Magnitudes of difference
229 between treatments were determined using the standardized mean difference (effect size), the
230 within-subject placebo standard deviation, and are expressed as Cohen's *d*. Chances of the true
231 value of the effect being possibly (≥75%), likely (≥95%), very likely (≥99.5%), or most likely
232 (=100%), were calculated according to established criteria (Hopkins et al. 2009), with clinical
233 chances of benefit or harm considered for running performance variables. An n=8 subjects
234 provided adequate statistical power in a post-only cross-over design assuming a reference
235 difference (between treatments) in work done of 7%, a typical error in repeat sprints of 4.5%,
236 and Type I and II errors of 5% and 20% respectively.
237
238 **Results**
239 **Running performance**: Participants ingesting a combination of caffeine + EAAs completed
240 more mean sprint work (+174 J, 23–324 J; mean difference, 95%CI; *d*=0.30, likely beneficial ;
241 Table 2) and mean sprint power (43 W, 6–81 W; *d*=0.30, likely beneficial), and had higher peak
242 sprint velocity (0.17 $m.s^{-1}$, 0.04–0.31 $m.s^{-1}$; *d*=0.50, very likely beneficial) compared to EAA's
243 alone (Table 2). Mean sprint work, power and velocity were greater in set 1 compared to all
244 other sets (Supplementary Table 1). There was no interaction effect between time and

supplement for any running performance variables. Total work performed excluding the sprints showed no significant differences between supplementation protocols.

Table 2: Comparison of mechanical data between supplementation strategies. Data are mean ± SD

	Placebo	Caffeine	LNAA	Caffeine + LNAA
Mean Sprint Work (J)	2705 ± 581	2791 ± 604	2678 ± 664	2858 ± 763*
Mean Sprint Power (W)	676 ± 145	698 ± 151	669 ± 166	715 ± 191*
Peak Sprint Velocity ($m.s^{-1}$)	5.78 ± 0.34	5.83 ± 0.35	5.76 ± 0.44	5.94 ± 0.37*
Total Work (Without Sprints) (J)	104894 ± 18359	108638 ± 15593	105047 ± 14985	103086 ± 18698

***** denotes statistical significant difference from LNAA where $p \leq 0.05$

Blood parameters: All supplementation strategies were effective in elevating protein and caffeine concentration (Figure 3, Supplementary table 2).

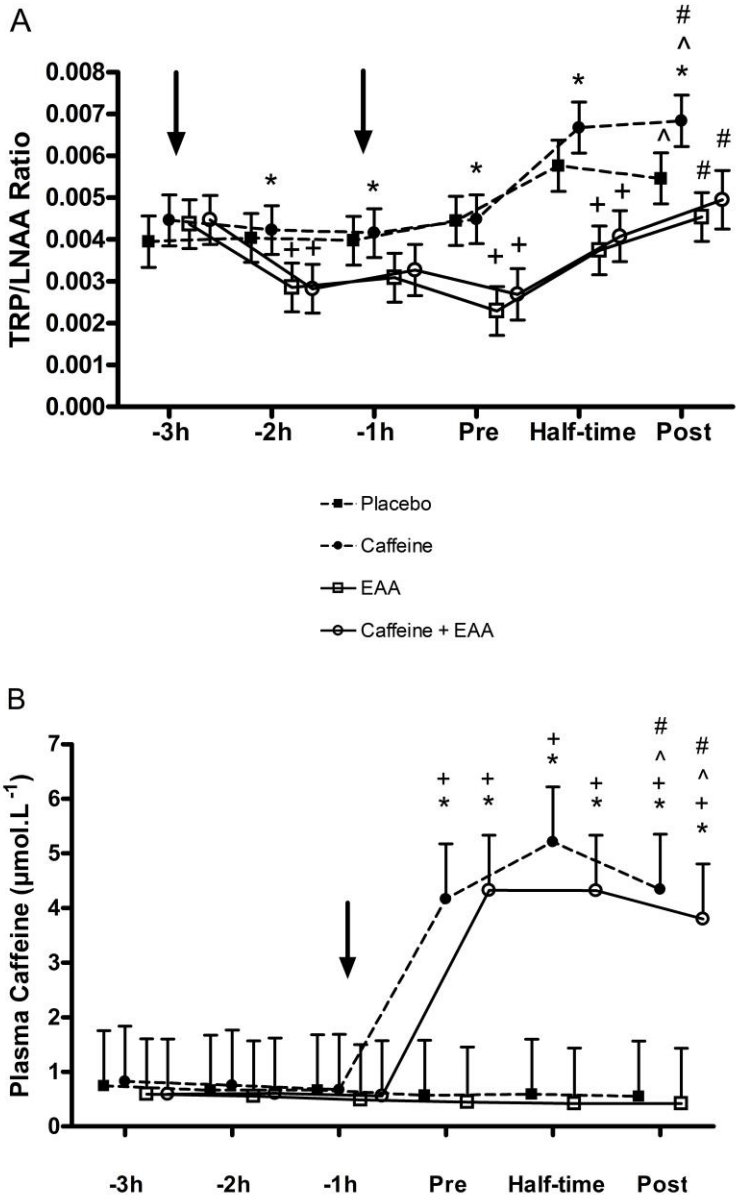
EAA: In particular, supplementing with EAAs reduced the plasma free tryptophan:LNAA ratio in the caffeine + EAA condition compared to placebo (0.0009, 0.0005–0.0013;*d*=0.76, most likely) and caffeine alone (0.0014, 0.0010–0.0018; *d*=1.21, most likely). There was an effect of time on the plasma free tryptophan:LNAA ratio consistent with ingestion timing.

Caffeine: As expected, plasma caffeine was increased in the caffeine only condition when compared to placebo (4.3 $\mu mol.L^{-1}$, 3.2–5.4 $\mu mol.L^{-1}$; *d*=2.65, most likely), and EAA only (4.4 $\mu mol.L^{-1}$, 3.4–5.5 $\mu mol.L^{-1}$; *d*=2.74, most likely). Plasma caffeine was also higher in the caffeine + EAA condition compared to placebo (4.7 $\mu mol.L^{-1}$, 3.6–5.7 $\mu mol.L^{-1}$; *d*=2.88, most likely) and

EAA alone (4.8 µmol.L^{-1}, 3.7-5.9 µmol.L^{-1}; *d*=2.97, most likely; Figure 3B) There was also an effect of time on caffeine concentration, relevant to when caffeine was ingested.

pO_2 & SO_2: Venous blood pO_2 was substantially higher in the caffeine + EAA treatment compared to caffeine alone (7.0 mmHg, 1.7–12.3mmHg; *d*=0.39, likely) and placebo (6.2 mmHg, 1.1–11.2 mmHg; 0.35, likely) across the exercise protocol (Supplementary Table 2). Oxygen saturation, as indicated by venous blood SO_2, increased from pre- to post-exercise by 37% (23-50%; *d*=2.28, most likely) across treatments (Supplementary Table 2).

Lactate: Blood lactate was also substantially higher (0.55 mmol L^{-1}, 0.15–0.95; *d*=0.17, possibly; Supplementary Table 2) in the caffeine + EAA group compared to a placebo across all time points.



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278 **Figure 3:** Comparison of Free Tryptophan:LNAA ratio and caffeine concentrations between
279 supplementation strategies. Data are estimated mean ± 95% CI. * denotes significant difference
280 from EAA at time point where $p \leq 0.05$. + denotes significant difference from placebo at time
281 point where $p \leq 0.05$. ^ denotes significant difference from EAA overall where $p \leq 0.05$. #
282 denotes significant difference from placebo overall where $p \leq 0.05$. ↓ indicates supplement
283 ingestion time.

MVC: Overall, MVC force was higher (31nM, 8–54nM; *d*=0.62, likely beneficial) when caffeine + EAA were ingested compared to a placebo (Figure 4A).

Peripheral Twitch: Peripheral twitch force was not different between supplementation protocols across all time points.

Central Activation Ratio and EMG: The decline in normalized EMG activity was attenuated with caffeine + EAA compared with placebo (19%, 9–29%; *d*=0.84, most likely beneficial) and EAA alone (13%, 0–26%; *d*=0.58, likely beneficial). Supplementing with 'caffeine only' resulted in a lower reduction in normalized EMG compared to placebo (15%, 1–29%; *d*=0.67, likely beneficial; Figure 4B). There was no effect of time and no interaction between time and supplement for decline in EMG activity (Supplementary Table 1). Ingesting a combination of caffeine + EAA resulted in a higher overall CAR (2.7%, -5.4 to -0.4%; *d*=0.5, likely beneficial; Figure 4C) compared to a placebo. There was no effect of time, and no interaction between time and supplement.

NIRS: Greater mean declines in TSI across the exercise protocol were observed when caffeine (-8.3%, 3.7–13.0%; *d*=0.9, most likely beneficial) and caffeine + EAA (-5.6%, 1.0–10.1%; *d*=0.6, very likely beneficial) were ingested (the two conditions that produced the highest amount of work), compared with EAAs alone (Figure 4D, Supplementary Table 1).

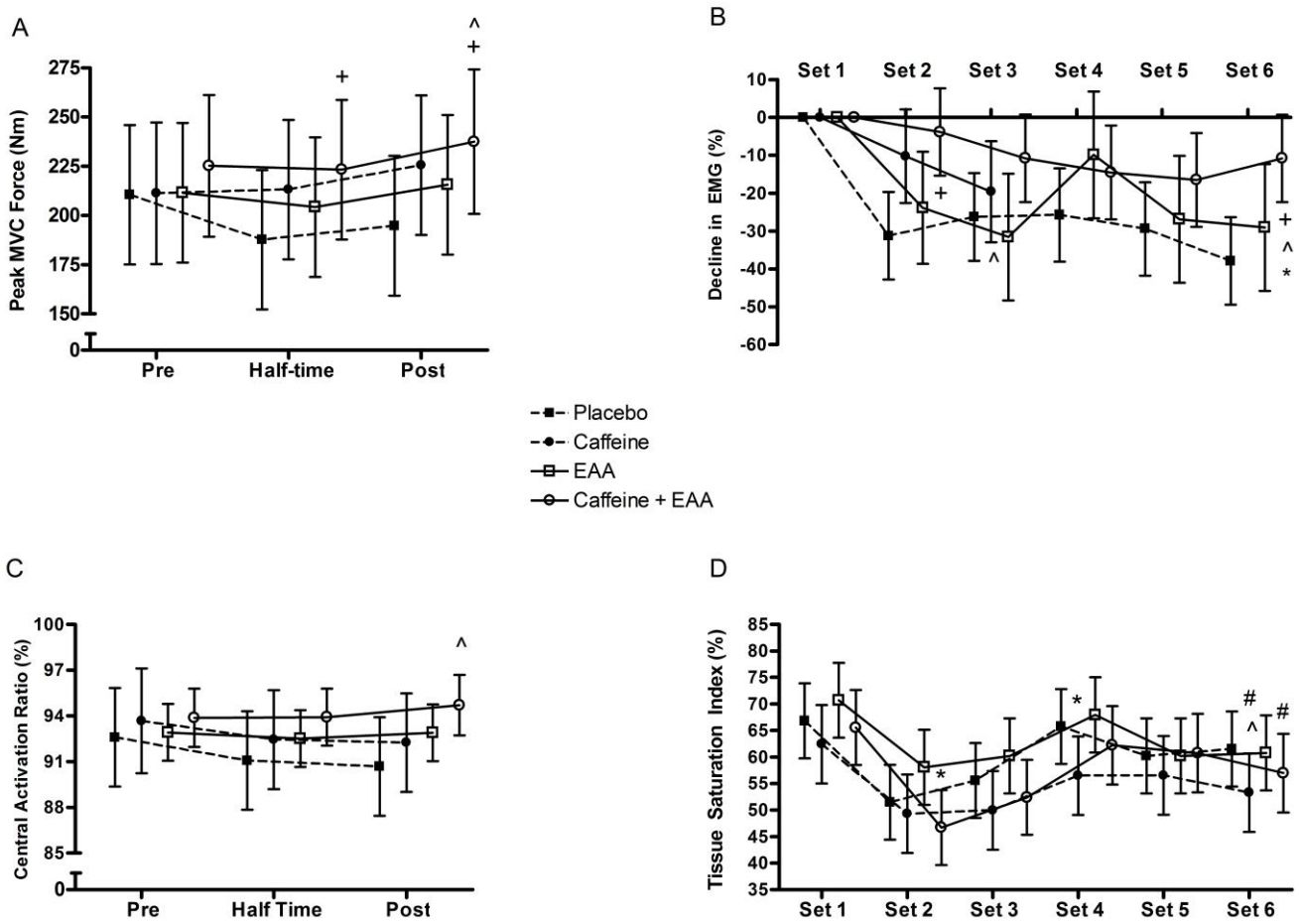

Figure 4: Comparison of central fatigue markers and total work (J) between supplementation strategies. Data are estimated mean ± 95%CI. * denotes significant difference from EAA at time point where $p \leq 0.05$. + denotes significant difference from placebo at time point where $p \leq 0.05$. ^ denotes significant difference from EAA overall where $p \leq 0.05$. # denotes significant difference from placebo overall where $p \leq 0.05$.

Discussion

This study demonstrated, for the first time, that nutritional supplementation with a combination of caffeine and EAAs attenuated the development of central fatigue in response to simulated team-sport exercise in hot and hypoxic environmental conditions. Using EMG activity and CAR as markers of muscle recruitment and central activation, respectively, supplementation with caffeine + EAA delayed the onset of central fatigue in team-sport players, but this effect did not translate into clear performance benefits.

Our supplementation protocol was designed to target two different neurotransmitter pathways to reduce central fatigue, based on previous literature (Pardridge 1998). Due to the challenges of measuring neurotransmitter concentrations *in vivo* in humans, we can only use a conceptual framework based on prior literature using EAA's containing LNAA's to manipulate neurotransmitters (Pardridge 1998). Our supplementation protocol used EAA's (including LNAA's) to inhibit serotonin synthesis as indicated by a lower free tryptophan:LNAA ratio (Figure 4A; (Pardridge 1998). Additionally, we aimed to promoting dopamine production in the CNS via phenylalanine ingestion (Pardridge 1998). We believe this was achieved, as indicated by the lower ratio of free tryptophan:LNAA and increased phenylalanine concentrations in the plasma when EAAs were ingested with or without caffeine (Figure 4A, Supplementary Table 3). We believe this effectively lowered the serotonin:dopamine ratio, which is considered important in attenuating central fatigue (Fernstrom 2013). Secondly, we aimed to antagonise adenosine receptors via caffeine supplementation (Figure 4, Supplementary Table 3). Isolated EAA and caffeine supplementation are known to effect central drive and motor unit recruitment in thermoneutral (Meeusen et al. 2006) and hot environments (Del Coso et al.

2008; Meeusen & Roelands 2010). Antagonizing adenosine or increasing dopamine synthesis has been linked to maintenance of motor unit recruitment and frequency of activation concurrent with improved exercise performance (Del Coso et al. 2008). It is surprising that only caffeine and caffeine + EAA had a clear influence on central fatigue variables. We consider that changes in markers of central drive (CAR and EMG) are likely due to caffeine, and a minor contribution from the EAA's in our combined supplementation protocol.

Physiological changes in central fatigue variables induced by caffeine and/or EAA, did not translate into a substantial performance benefit. Participants who ingested a combination of caffeine and EAAs performed more work during sprints than with EAA alone, but not significantly more than with a placebo. This is surprising given that supplementation with 3–6mg.kg^{-1} of caffeine has previously shown to improve sprint times and repeated-sprint performance (Glaister et al. 2008; Gant et al. 2010) compared to a placebo. While EAA supplementation may not improve performance in normal conditions, there is evidence it is beneficial when exercise is performed in the heat (Mittleman et al. 1998; Tumilty et al. 2011). However, the effect of EAA supplementation on high-intensity exercise performance is unknown. Our findings provide new insight into the influence of isolated and combined supplementation protocols on repeat-sprint performance.

Cerebral oxygenation impairs central drive and motor unit recruitment during repeated-sprint performance in hypoxic conditions (Smith & Billaut 2010; Billaut et al. 2013), and other forms of high-intensity exercise (Rooks et al. 2010). Caffeine ingestion may reduce cerebral blood flow and oxygen availability (Kennedy & Haskell 2011), which could explain the observed reductions

in cerebral oxygenation in the final sets of sprints. However, our data appear to dissociate cerebral oxygenation as a mechanism for central fatigue in hot and hypoxic conditions. We consider that reduced cerebral oxygenation observed in the caffeine only and caffeine + EAA trials is a likely consequence of caffeine ingestion and/or the somewhat greater mechanical work produced.

To our knowledge, this is the first study to measure central fatigue via EMG and magnetic stimulation in conjunction with nutritional supplementation. Although we used a simulated exercise protocol and unique environmental conditions, we believe our data provide novel insights into central fatigue and match performance. This study highlights the potential for nutritional interventions to sustain or enhance exercise performance in challenging environmental conditions. We observed large standard deviations in our performance data, which may be responsible for the lack of significant difference between caffeine + EAA and placebo supplementation protocols. Furthermore, the dose of caffeine used in the study is at the bottom of the recommended range for ergogenic benefit, which may explain why we observed no performance improvements with caffeine supplementation alone. We acknowledge that there may also have been an unaccounted for interaction between pre-exercise carbohydrate feeding and AA supplement on performance. Finally, it is plausible that the environmental conditions chosen for the study induced a level of fatigue that was too great for supplementation alone to overcome, again contributing to the lack of running performance improvements. The observed changes in central fatigue variables may have been too small to enhance performance and these relationships warrant further investigation.

In conclusion, team-sport athletes who ingest a combination of caffeine and EAA may attenuate central fatigue and perform more work than when ingesting EAA's alone during repeated sprint exercise in hot, hypoxic conditions. The mechanisms explaining this improved performance appear to be associated with central drive and muscle activation, and, possibly, altered neurotransmitter production or function influencing the motivation to exercise. Further work should evaluate similar supplementation and exercise protocols in thermoneutral and normoxic conditions. This line of investigation would help establish the effectiveness of combining supplements for team-sport athletes in more common match and training environments.

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Supplementary Table 1. Individual time point data for performance outcome variables (6 time points) Data are mean ± SD

Variable	Condition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Mean sprint work (J)	**Placebo**	2860 ± 458	2719 ± 466	2510 ± 766	2819 ± 567	2599 ± 638	2722 ± 657
	Caffeine	2940 ± 484	2775 ± 687	2829 ± 664	2811 ± 610	2686 ± 671	2708 ± 660
	LNAA	2911 ± 530	2620 ± 798	2513 ± 826	2820 ± 595	2525 ± 680	2679 ± 634
	Caffeine + LNAA	2974 ± 677	2888 ± 860	2837 ± 814	2858 ± 781	2742 ± 821	2867 ± 868
Mean sprint power (W)	**Placebo**	715 ± 115	680 ± 117	628 ± 191	705 ± 142	650 ± 159	681 ± 164
	Caffeine	735 ± 121	694 ± 172	707 ± 166	703 ± 152	672 ± 167	677 ± 165
	LNAA	728 ± 132	655 ± 199	628 ± 206	705 ± 149	631 ± 170	670 ± 158
	Caffeine + LNAA	743 ± 169	722 ± 215	709 ± 203	715 ± 195	686 ± 205	717 ± 217
Peak sprint velocity ($m.s^{-1}$)	**Placebo**	5.71 ± 0.35	5.78 ± 0.29	5.79 ± 0.41	5.74 ± 0.35	5.69 ± 0.40	5.97 ± 0.34
	Caffeine	5.81 ± 0.26	5.77 ± 0.32	6.00 ± 0.30	5.64 ± 0.44	5.75 ± 0.39	6.02 ± 0.29
	LNAA	5.79 ± 0.32	5.73 ± 0.47	5.79 ± 0.50	5.68 ± 0.47	5.63 ± 0.50	5.92 ± 0.45
	Caffeine + LNAA	5.93 ± 0.29	5.91 ± 0.30	6.00 ± 0.39	5.79 ± 0.30	5.87 ± 0.44	6.13 ± 0.46
Mean EMG decline (%)	**Placebo**	100 ± 0	31.5 ± 31.1	26.5 ± 9.2	26.7 ± 21.4	30.0 ± 17.7	38.2 ± 30.2
	Caffeine	100 ± 0	9.9 ± 9.7	20.9 ± 6.6			
	LNAA	100 ± 0	25.2 ± 7.1	27.7 ± 4.3	11.8 ± 9.1	28.6 ± 4.0	30.2 ± 10.2
	Caffeine + LNAA	100 ± 0	4.9 ± 4.9	11.8 ± 5.8	15.4 ± 10.5	17.0 ± 8.5	11.9 ± 10.4
Tissue saturation index (%)	**Placebo**	66.8 ± 6.2	51.5 ± 4.9	55.6 ± 9.2	65.8 ± 7.1	60.2 ± 9.2	61.5 ± 9.8
	Caffeine	62.4 ± 9.3	49.1 ± 10.8	49.8 ± 11.8	56.4 ± 9.7	56.5 ± 10.5	53.2 ± 14.5
	LNAA	70.7 ± 8.3	58.1 ± 10.0	60.2 ± 9.3	67.9 ± 6.9	60.2 ± 9.4	60.8 ± 9.6
	Caffeine + LNAA	65.6 ± 9.5	46.7 ± 12.7	52.4 ± 7.9	62.3 ± 11.5	60.8 ± 16.9	57.1 ± 11.6

Supplementary Table 2. Venous pCO_2, pO_2, sO_2, lactate, and glucose concentrations between supplementation strategies. Data are mean ± SD

Variable	Condition	3hr pre	2hr pre	1hr pre	Pre	Half time	Post
pCO_2 (mmHg)	**Placebo**	57 ± 7	59 ± 4	62 ± 5	54 ± 9	33 ± 4	30 ± 5
	Caffeine	53 ± 9	55 ± 9	60 ± 5	56 ± 9	32 ± 7	30 ± 6
	LNAA	56 ± 7	57 ± 5	59 ± 4	50 ± 7	31 ± 6	31 ± 6
	Caffeine + LNAA*	59 ± 8	56 ± 8	57 ± 9	51 ± 8	28 ± 4	26 ± 3
pO_2 (mmHg)	**Placebo**	31 ± 4	24 ± 3	20 ± 4	27 ± 10	53 ± 14	63 ± 9
	Caffeine	34 ± 8	27 ± 12	20 ± 4	24 ± 8	55 ± 17	54 ± 17
	LNAA	33 ± 11	24 ± 4	20 ± 4	33 ± 9	64 ± 13	60 ± 10
	Caffeine + LNAA*^	33 ± 9	29 ± 8	24 ± 9	30 ± 10	65 ± 4	71 ± 3
sO_2 (%)	**Placebo**	52 ± 11	39 ± 10	34 ± 12	46 ± 23	82 ± 12	89 ± 8
	Caffeine	65 ± 18	45 ± 23	31 ± 10	43 ± 19	80 ± 16	79 ± 16
	LNAA	58 ± 20	40 ± 12	30 ± 12	60 ± 19	88 ± 10	87 ± 8
	Caffeine + LNAA*^	60 ± 13	51 ± 17	41 ± 20	53 ± 21	90 ± 1	92 ± 1
Lactate (mmol/L)	**Placebo**	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	7.1 ± 1.9	7.3 ± 1.9
	Caffeine	1.1 ± 0.5	0.9 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	7.4 ± 2.0	8.5 ± 1.8
	LNAA	1.2 ± 0.7	0.9 ± 0.3	0.9 ± 0.6	1.2 ± 0.5	7.5 ± 1.9	7.1 ± 1.5
	Caffeine + LNAA*	0.9 ± 0.3	0.8 ± 0.1	0.9 ± 0.5	1.3 ± 0.9	8.2 ± 2.4	9.4 ± 2.4
Glucose (mmol/L)	**Placebo**	4.0 ± 0.9	4.1 ± 0.6	4.0 ± 0.4	4.7 ± 0.8	5.9 ± 0.9	5.6 ± 1.7
	Caffeine	4.5 ± 0.5	4.5 ± 0.8	4.5 ± 1.1	4.6 ± 0.7	6.1 ± 1.9	6.8 ± 1.8
	LNAA^	4.5 ± 1.0	3.7 ± 0.3	4.4 ± 0.8	4.3 ± 0.5	6.3 ± 1.5	5.7 ± 1.3
	Caffeine + LNAA	3.9 ± 1.0	3.7 ± 0.3	4.2 ± 1.1	4.6 ± 0.9	6.8 ± 2.2	6.3 ± 1.8

* denotes significant difference from placebo treatment over all time points where $p \leq 0.05$

^ denotes significant difference from caffeine treatment over all time points where $p \leq 0.05$

Supplementary Table 3. Change in individual LNAA concentrations between supplementation strategies. Data are mean ± SD

Variable	Condition	3hr pre	2hr pre	1hr pre	Pre	Half time	Post
Histidine (µmol/L)	**Placebo*^**	82 ± 6	78 ± 6	78 ± 5	75 ± 8	82 ± 10	77 ±112
	Caffeine	80 ± 5	83 ± 7	81 ± 9	76 ± 10	77 ± 10	85 ± 9
	LNAA#	80 ± 9	104 ± 11	84 ± 9	109 ± 21	87 ± 9	85 ± 7
	Caffeine + LNAA	84 ± 12	119 ± 23	90 ± 11	116 ± 17	94 ± 14	91 ± 13
Arginine (µmol/L)	**Placebo***	92 ± 9	84 ± 11	84 ± 9	76 ± 11	83 ± 15	81 ± 19
	Caffeine*	91 ± 14	90 ± 14	87 ± 13	77 ± 11	74 ± 12	82 ± 11
	LNAA	90 ± 19	124 ± 18	105 ± 14	139 ± 39	103 ± 15	96 ± 16
	Caffeine + LNAA	95 ± 16	145 ± 24	110 ± 11	138 ± 23	105 ± 9	91 ± 6
Threonine (µmol/L)	**Placebo*^**	129 ± 23	122 ± 22	116 ± 15	108 ± 18	110 ± 17	105 ± 24
	Caffeine*^	132 ± 29	131 ± 26	126 ± 22	116 ± 28	105 ± 20	115 ± 25
	LNAA	128 ± 32	141 ± 23	125 ± 19	153 ± 37	122 ± 18	121 ± 15
	Caffeine + LNAA*	136 ± 22	163 ± 24	137 ± 17	157 ± 32	129 ± 20	122 ± 16
Lysine (µmol/L)	**Placebo*^**	167 ± 26	151 ± 21	146 ± 17	134 ± 22	147 ± 22	132 ± 27
	Caffeine*^	170 ± 40	166 ± 30	158 ± 27	139 ± 33	126 ± 20	136 ± 16
	LNAA*	165 ± 51	196 ± 36	165 ± 31	200 ± 52	150 ± 28	136 ± 24
	Caffeine + LNAA	178 ± 48	238 ± 57	179 ± 22	211 ± 40	159 ± 24	143 ± 22

Methionine (µmol/L)	**Placebo***	28 ± 6	25 ± 5	23 ± 4	22 ± 4	24 ± 4	26 ± 6
	Caffeine	32 ± 6	29 ± 4	27 ± 4	24 ± 6	24 ± 5	28 ± 4
	LNAA	28 ± 7	34 ± 6	28 ± 4	34 ± 10	28 ± 4	28 ± 4
	Caffeine + LNAA	32 ± 7	40 ± 7	28 ± 5	34 ± 8	28 ± 5	27 ± 3
Valine (µmol/L)	**Placebo*^**	255 ± 33	249 ± 62	241 ± 46	237 ± 33	240 ± 36	237 ± 49
	Caffeine*^	257 ± 25	245 ± 28	240 ± 44	220 ± 21	211 ± 26	224 ± 43
	LNAA*	250 ± 46	320 ± 41	284 ± 22	372 ± 72	305 ± 40	285 ± 36
	Caffeine + LNAA	280 ± 45	391 ± 67	320 ± 50	402 ± 47	324 ± 38	301 ± 47
Isoleucine (µmol/L)	**Placebo*^**	65 ± 15	60 ± 25	58 ± 17	62 ± 11	62 ± 14	65 ± 24
	Caffeine*^	72 ± 13	62 ± 12	63 ± 21	55 ± 10	55 ± 8	61 ± 12
	LNAA	65 ± 17	100 ± 17	82 ± 19	122 ± 30	87 ± 14	77 ± 20
	Caffeine + LNAA	78 ± 15	125 ± 32	87 ± 24	121 ± 24	89 ± 21	69 ± 20
Leucine (µmol/L)	**Placebo*^**	129 ± 23	118 ± 34	113 ± 25	116 ± 17	118 ± 19	120 ± 37
	Caffeine*^	140 ± 25	125 ± 21	123 ± 35	108 ± 18	106 ± 13	116 ± 22
	LNAA	126 ± 29	179 ± 25	151 ± 25	213 ± 50	160 ± 23	142 ± 29
	Caffeine + LNAA	148 ± 8	222 ± 52	162 ± 38	220 ± 38	161 ± 27	136 ± 33

Phenylalanine (µmol/L)	**Placebo*^**	57 ± 7	52 ± 7	51 ± 8	52 ± 7	55 ± 7	57 ± 10
	Caffeine*^	60 ± 10	58 ± 9	55 ± 10	52 ± 7	54 ± 6	60 ± 9
	LNAA*	55 ± 11	81 ± 14	73 ± 15	102 ± 23	88 ± 18	86 ± 12
	Caffeine + LNAA	62 ± 13	99 ± 24	75 ± 4	112 ± 22	96 ± 18	93 ± 14
Tryptophan (µmol/L)	**Placebo^+**	4.0 ± 0.7	3.8 ± 0.8	3.6 ± 0.8	3.9 ± 0.6	5.1 ± 1.8	5.4 ± 1.0
	Caffeine^	4.6 ± 0.4	4.2 ± 0.7	4.0 ± 1.0	3.9 ± 0.4	5.5 ± 1.2	6.2 ± 0.8
	LNAA*	4.3 ± 0.7	3.6 ± 0.5	3.4 ± 0.9	3.3 ± 1.1	4.2 ± 1.1	4.8 ± 1.2
	Caffeine + LNAA*	4.9 ± 0.8	4.3 ± 0.8	3.9 ± 0.7	4.1 ± 1.4	4.9 ± 0.8	5.2 ± 0.8

* denotes significant difference between treatment and caffeine + LNAA treatment over all time points where $p \leq 0.05$

^ denotes significant difference between treatment and LNAA treatment over all time points where $p \leq 0.05$

+ denotes significant difference between treatment and caffeine treatment over all time points where $p \leq 0.05$

# denotes significant difference between treatment and placebo treatment over all time points where $p \leq 0.05$

Supplementary Table 4. Individual time point data (3 time points) for main outcome variables. Data are mean ± SD

Variable	Condition	Pre	Half-Time	Post
Mean MVC force (nM)	**Placebo**	211 ± 52	188 ± 48	195 ± 53
	Caffeine	218 ± 43	213 ± 44	226 ± 62
	LNAA	212 ± 46	204 ± 36	216 ± 40
	Caffeine + LNAA	215 ± 35	223 ± 41	231 ± 33
Mean twitch force (nM)	**Placebo**	68.5 ± 21.6	62.7 ± 22.9	71.2 ± 24.5
	Caffeine	68.1 ± 19.3	65.8 ± 22.9	69.5 ± 15.6
	LNAA	68.9 ± 20.9	65.0 ± 17.5	66.1 ± 14.5
	Caffeine + LNAA	62.8 ± 17.4	59.5 ± 12.2	71.0 ± 16.3
Central activation ratio (%)	**Placebo**	92.6 ± 2.9	91.1 ± 5.3	90.7 ± 7.6
	Caffeine	93.5 ± 1.5	92.5 ± 2.5	92.3 ± 5.5
	LNAA	92.9 ± 4.2	92.5 ± 4.7	92.9 ± 4.5
	Caffeine + LNAA	94.5 ± 2.0	93.9 ± 3.8	96.1 ± 2.2